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NES-1 POLYPEPTIDES, DNA, AND RELATED MOLECULES AND METHODS
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MDA-468(BT) MDA-436(BT 39VTE7(NEI) 21MT-1(BT 76R30(RT 4VNF(NF) 4VN(NE)

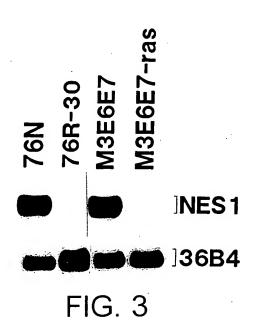
T47-D(BT 7VNF(NF) 76N(NE) 3VN(NE) FS-2(F)

NES1[

MDA-231(BT) MDA-435(BT) MDA-157(BT) MCF-10A(NI) ZR-75-1(BT) HBL-100(MI) HS578T(BT) M2E6E7(MI) M3E6E7(MI) MCF-7(BT) 7VNE6(NI) **6VNF(NF) 76NF(NF)** Caski(CT) Hela(CT) 3VN(NE) Siha(CT) NES₁



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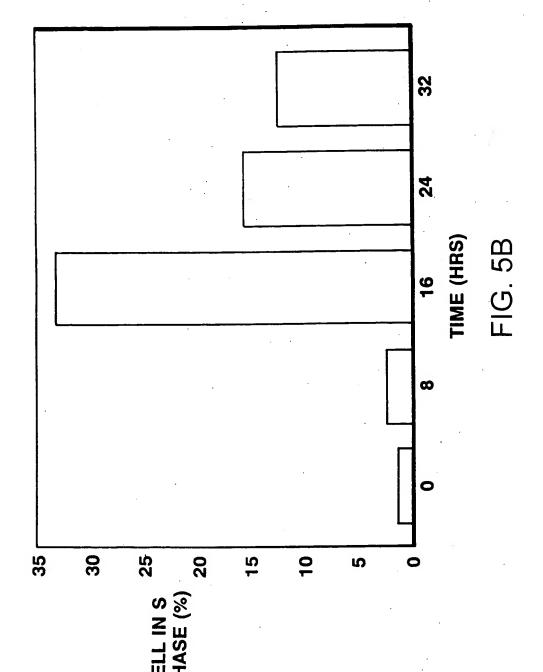




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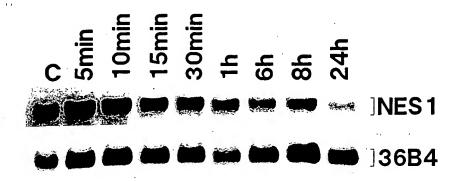


FIG. 7A



c PMA 1 2 NES1 36B4 FIG. 8



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Comparison of Sequences near putative active site

NES 1	86 86	7 137	D 223	J 224		5 230 D 230		D 241	- 243	245	
Human Pancreatic	н	D	D	s		G	*	G	v	w	
Trypsinogen III	A	A	Δ	Δ	1	\		Δ	Δ	Δ	

A: Catalytic triad

 \triangle : Residues important for substrate binding and specificity

FIG. 9A

Alignment of NES 1 amino acid sequence with other serine proteases

	1			•	50
Mmtryar	MSALLILA	LVGA		AVAFPVDD.D	DKIVGGY
Hstryivb	LELHP	LLGGRTWRAA	RDADGCEALG	TVAVPFDD.D	DKIVGGY
Rntrypvb	MKICIFFT	LLGT		VAAFPTEDND	DRIVGGY
Sstrypii				.AAFATED	DKIVGGY
Nesl	MRAPHLHLSA	ASGARALAKL	LPLLMAQLWA	AEAALLPQND	TRLDPEAYGA
				-	
	51				100
Mmtryar	TCRESSVPYQ	VSLNAGYHF.	CGGSLINDQW	VVSAAHCYKY	RIQVRLGEHN
Hstryivb	TC. ENSLPYQ	VSLNSGSHF.	CGGSLISEQW	VVSAAHCYKT	RIQURLGEHN
Rntrypvb	TCQEHSVPYQ	VSLNAGSHI.	CGGSLITDQW	VLSAAHCYHP	QLQVRLGEHN
Sstrypii	ECKAYSQPHQ	VSLNSGYHF.	CGGSLVNENW	VVSAAHCYQS	RVEVRLGEHN
Nesl	PCARGSQPWQ	VSLFNGLSFH	CAGVLVDQSW	VLTAAHCGNK	PLWARVGDDH
	101	•	. ,		150
Mmtryar	INVLEGNEQF	VDSAKIIRHP	NYN	.SWTLDNDIM	LIKLASPVTL
Hstryivb	IKVLEGNEQF	INAAKIIRHP	KYN	.RDTLDNDIM	LIKLSSPAVI
Rntrypvb	IYEIEGAEQF	IDAAKMILHP	DYD	.KWTVDNDIM	LIKLKSPATL
Sstrypii	IQVTEGSEQF	ISSSRVIRHP	NYS	.SYNIDNDIM	LIKLSKPATL
Nesl	LLLLQG.EQL	RRTTRSVVHP	KYHQGSGPIL	PRRTDEHDLM	LLKLARPVVP
			L		
			EVTDA INICE	20 ←	
	151		EXTRA INSE	ERT -	200
Mmtryar	151 NARVASVPLP	SSCAPAGTQC		NGVNNPDLLQ	
Mmtryar Hstryivb		SSCAPAGTQC TAPPAAGTEC	LISGWGNTLS		CVDAPVLPQA
-	NARVASVPLP		LISGWGNTLS LISGWGNTLS	NGVNNPDLLQ	CVDAPVLPQA CLDAPVLTQA
Hstryivb	NARVASVPLP NARVSTISLP	TAPPAAGTEC	LISGWGNTLS LISGWGNTLS LVSGWG.VLK	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS
Hstryivb Rntrypvb	NARVASVPLP NARVSTISLP NSKVSTIPLP	TAPPAAGTEC QYCPTAGTEC	LISGWGNTLS LISGWGNTLS	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS
Hstryivb Rntrypvb Sstrypii	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS
Hstryivb Rntrypvb Sstrypii	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP 201	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS
Hstryivb Rntrypvb Sstrypii	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK
Hstryivb Rntrypvb Sstrypii Nesl	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP 201	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA EGGKDSCQGD	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK 250
Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP 201 DCEASYPGDI	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC TNNMICVGFL	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA EGGKDSCQGD EGGKDSCQGD	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK 250 LQGIVSWG.Y
Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar Hstryivb	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP 201 DCEASYPGDI ECKASYPGKI	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC TNNMICVGFL TNSMFCVGFL	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA EGGKDSCQGD EGGKDSCQGD EGGKDSCQRD	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK 250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D
Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar Hstryivb Rntrypvb	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP 201 DCEASYPGDI ECKASYPGKI VCHKAYPRQI	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC TNNMICVGFL TNSMFCVGFL TNNMFCLGFL	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK 250 LQGIVSWG.Y LQGVVSWG.H
Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar Hstryivb Rntrypvb Sstrypii	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP 201 DCEASYPGDI ECKASYPGKI VCHKAYPRQI DCNNSYPGMI	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC TNNMICVGFL TNSMFCVGFL TNNMFCLGFL TNAMFCAGYL	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK 250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D LQGVVSWG.Y
Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar Hstryivb Rntrypvb Sstrypii	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP 201 DCEASYPGDI ECKASYPGKI VCHKAYPRQI DCNNSYPGMI	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC TNNMICVGFL TNSMFCVGFL TNNMFCLGFL TNAMFCAGYL	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD DRGQDPCQSD	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK 250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D LQGVVSWG.Y LQGILSWGVY
Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar Hstryivb Rntrypvb Sstrypii	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP 201 DCEASYPGDI ECKASYPGKI VCHKAYPRQI DCNNSYPGMI ECEVFYPGVV	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC TNNMICVGFL TNSMFCVGFL TNNMFCLGFL TNAMFCAGYL	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD DRGQDPCQSD	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK 250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D LQGVVSWG.Y LQGILSWGVY
Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar Hstryivb Rntrypvb Sstrypii Nes1	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP 201 DCEASYPGDI ECKASYPGKI VCHKAYPRQI DCNNSYPGMI ECEVFYPGVV 251	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC TNNMICVGFL TNSMFCVGFL TNNMFCLGFL TNAMFCAGYL TNNMICAG.L	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD DRGQDPCQSD AAA	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK 250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D LQGVVSWG.Y LQGILSWGVY
Mmtryar Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar Hstryivb Rntrypvb Sstrypii Nes1	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP 201 DCEASYPGDI ECKASYPGKI VCHKAYPRQI DCNNSYPGMI ECEVFYPGVV 251 GCAQPDAPGV	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC TNNMICVGFL TNSMFCVGFL TNNMFCLGFL TNAMFCAGYL TNNMICAG.L YTKVCNYVDW	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD DRGQDPCQSD 280 IQNTIADN*.	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK 250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D LQGVVSWG.Y LQGILSWGVY
Mmtryar Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP 201 DCEASYPGDI ECKASYPGKI VCHKAYPRQI DCNNSYPGMI ECEVFYPGVV 251 GCAQPDAPGV GCAWKNRPGV	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC TNNMICVGFL TNSMFCVGFL TNMFCLGFL TNAMFCAGYL TNMMICAG.L YTKVCNYVDW YTKVYNYVDW	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA EGGKDSCQGD EGGKDSCQGD EGGKDSCQGD DRGQDPCQSD 280 IQNTIADN*. IKDTIAANS*	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK 250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D LQGVVSWG.Y LQGILSWGVY
Mmtryar Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar Hstryivb Rntrypvb Sstrypii Nes1 Mmtryar Hstryivb Rntryar	NARVASVPLP NARVSTISLP NSKVSTIPLP NTYVQPVALP GPRVRALQLP 201 DCEASYPGDI ECKASYPGVI VCHKAYPRQI DCNNSYPGMI ECEVFYPGVV 251 GCAQPDAPGV GCAWKNRPGV GCALEGKPGV	TAPPAAGTEC QYCPTAGTEC TSCAPAGTMC YRCAQPGDQC TNNMICVGFL TNSMFCVGFL TNMFCLGFL TNMFCAGYL TNMMICAG.L YTKVCNYVDW YTKVYNYVDW YTKVYNYVDW YTKVCNYLNW YAKVCIFNDW	LISGWGNTLS LISGWGNTLS LVSGWG.VLK TVSGWGNTMS QVAGWGTTAA EGGKDSCQGD EGGKDSCQRD EGGKDSCQRD EGGKDSCQRD DRGQDPCQSD 280 IQNTIADN*. IKDTIAANS* IQQTVAAN*.	NGVNNPDLLQ FGADYPDELK FGFESPSVLQ STAD. KNKLQ RRVKYNKGLT SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE SGGPVVCNGE	CVDAPVLPQA CLDAPVLTQA CLDAPVLSDS CLNIPILSYS CSSITILSPK 250 LQGIVSWG.Y LQGVVSWG.H VQGIVSWG.D LQGVVSWG.Y LQGILSWGVY



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1 MRAPHLHLSA ASGARALAKL LPLLMAQLWA AEAALLPQND TRLDPEAYGA
51 PCARGSQPWQ VSLFNGLSFH CAGVLVDQSW VLTAAHCGNK PLWARVGDDH
101 LLLLQGEQLR RTTRSVVHPK YHQGSGPILP RRTDEHDLML LKLARPVVPG
151 PRVRALQLPY RCAQPGDQCQ VAGWGTTAAR RVKYNKGLTC SSITILSPKE
201 CEVFYPGVVT NNMICAGLDR GQDPCQSDSG GPLVCDETLQ GILSWGVYPC
251 GSAQHPAVYT QICKYMSWIN KVIRSN* (SEQ ID NO: 1)

AUG 2 5 7008

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CCTTCCTATC GGCGACTCCC AGATCCTGGC CATGAGAGCT CCGCACCTCC 51 ACCTCTCCGC CGCCTCTGGC GCCCGGGCTC TGGCGAAGCT GCTGCCGCTG 101 CTGATGGCGC AACTCTGGGC CGCAGAGGCG GCGCTGCTCC CCCAAAACGA 151 201 CACGCGCTTG GACCCCGAAG CCTATGGCGC CCCGTGCGCG CGCGGCTCGC AGCCCTGGCA GGTCTCGCTC TTCAACGGCC TCTCGTTCCA CTGCGCGGGT 251 GTCCTGGTGG ACCAGAGTTG GGTGCTGACG GCCGCGCACT GCGGAAACAA 301 351 GCCACTGTGG GCTCGAGTAG GGGATGATCA CCTGCTGCTT CTTCAGGGCG AGCAGCTCCG CCGGACGACT CGCTCTGTTG TCCATCCCAA GTACCACCAG 401 451 GGCTCAGGCC CCATCCTGCC AAGGCGAACG GATGAGCACG ATCTCATGTT GCTAAAGCTG GCCAGGCCCG TAGTGCCGGG GCCCCGCGTC CGGGCCCTGC 501 551 AGCTTCCCTA CCGCTGTGCT CAGCCCGGAG ACCAGTGCCA GGTTGCTGGC 601 TGGGGCACCA CGGCCGCCG GAGAGTGAAG TACAACAAGG GCCTGACCTG 651 CTCCAGCATC ACTATCCTGA GCCCTAAAGA GTGTGAGGTC TTCTACCCTG 701 GCGTGGTCAC CAACAACATG ATATGTGCTG GACTGGACCG GGGCCAGGAC 751 CCTTGCCAGA GTGACTCTGG AGGCCCCCTG GTCTGTGACG AGACCCTCCA AGGCATCCTC TCGTGGGGTG TTTACCCCTG TGGCTCTGCC CAGCATCCAG 801 CTGTCTACAC CCAGATCTGC AAATACATGT CCTGGATCAA TAAAGTCATA 851 CGCTCCAACT GATCCAGATG CTACGCTCCA GCTGATCCAG ATGTTATGCT 901 951 CCTGCTGATC CAGATGCCCA GAGGCTCCAT CGTCCATCCT CTTCCTCCCC 1001 AGTCGGCTGA ACTCTCCCCT TGTCTGCACT GTTCAAACCT CTGCCGCCCT 1051 CCACACCTCT AAACATCTCC CCTCTCACCT CATTCCCCCA CCTATCCCCA 1101 TTCTCTGCCT GTACTGAAGC TGAAATGCAG GAAGTGGTGG CAAAGGTTTA TTCCAGAGAA GCCAGGAAGC CGGTCATCAC CCAGCCTCTG AGAGCAGTTA 1151 1201 CTGGGGTCAC CCAACCTGAC TTCCTCTGCC ACTCCCGCT GTGTGACTTT 1251 GGGCAAGCCA AGTGCCCTCT CTGAACCTCA GTTTCCTCAT CTGCAAAATG 1301 GGAACAATGA CGTGCCTACC TCTTAGACAT GTTGTGAGGA GACTATGATA 1351 TAACATGTGT ATGTAAATCT TCATGTGATT GTCATGTAAG GCTTAACACA GTGGGTGGTG AGTTCTGACT AAAGGTTACC TGTTGTCGTG AAAAAAAAA 1401

1451 AAAA (SEQ ID NO: 2)

FIG. 11.